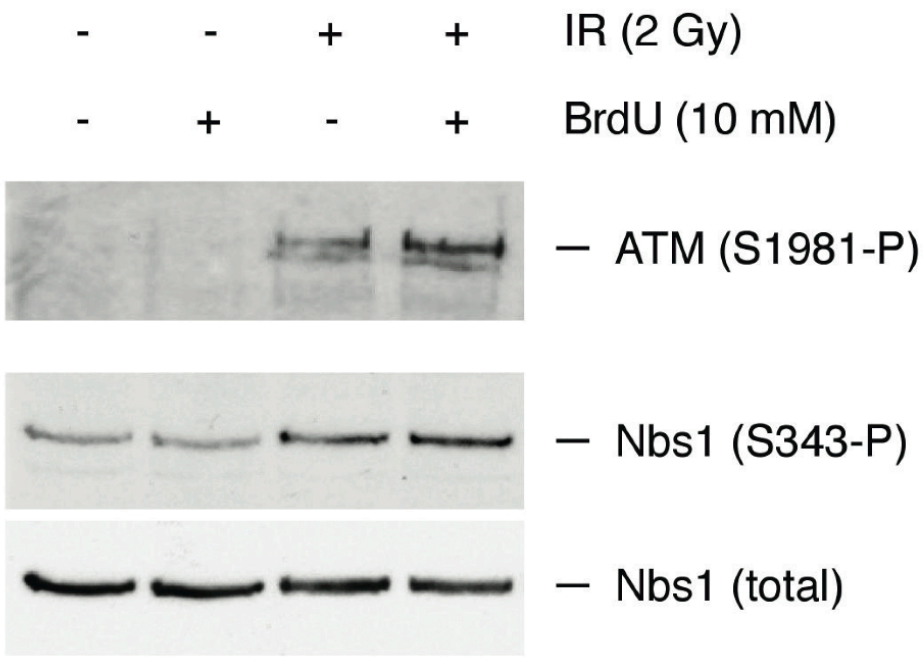
A



B

Laser output (%)	Mdc1 and Nbs1 recruitment to locally irradiated regions	
	Mock	BrdU (10 μm; 24 h)
40	-	-
45	-	+/-
50	-	+
55	-	+++
60	+/-	global

Legend to Supplementary information 6

Pre-senzitization with BrdU does not elicit a measurable checkpoint response on its own but determines the ability of UVA light (λ=337 nm) to generate spatially restricted DSBs. **(A)** U-2-OS cells were either left untreated or incubated with BrdU in the culture medium (10 μM final concentration) for 24 h. Where indicated, the cells were also subjected to ionizing radiation (IR; 2 Gy) 1 h before harvesting. The cell lysates were subsequently analyzed by immunoblotting with phospho-specific antibodies to ATM (S1981) and Nbs1 (S343) respectively. Note the lack of measurable signs of checkpoint activation in unirradiated, BrdU-treated cells. Total levels of Nbs1 serve as a loading control. **(B)** U-2-OS cells were pre-incubated with BrdU as in (A) or left untreated, and miroirradiated with the indicated output powers of the UVA laser (see Methods). The efficiency of generating DSBs spatially restricted to nuclear volumes exposed to the laser beam was assessed by immunochemically detectable accumulation of endogenous Mdc1 and/or Nbs1 within the microirradiated areas. Several independent experiments performed during the first 20 min after microirradiation consistently revealed that the laser output set to 50% produced the minimal energy necessary and sufficient to generate discernible local DSB areas in every pre-sensitized cell. Without BrdU pre-sensitization, these settings did not produce any discernible DNA damage. The boxed red values highlight the laser settings used throughout this study.